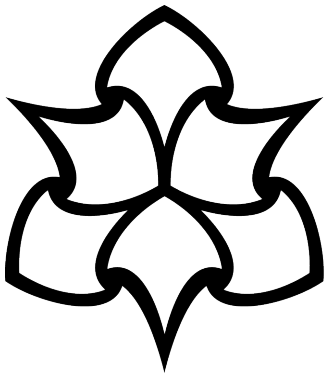
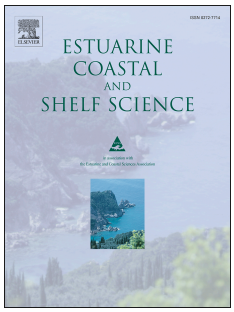


Journal Pre-proof



Mating system variation in neotropical black mangrove, *Avicennia germinans*, at three spatial scales towards an expanding northern distributional limit

John Paul Kennedy, Joshua M. Sammy, Jennifer K. Rowntree, Richard F. Preziosi

PII: S0272-7714(19)31091-1

DOI: https://doi.org/10.1016/j.ecss.2020.106712

Reference: YECSS 106712

To appear in: *Estuarine, Coastal and Shelf Science*

Received Date: 5 December 2019

Revised Date: 28 January 2020

Accepted Date: 11 March 2020

Please cite this article as: Kennedy, J.P., Sammy, J.M., Rowntree, J.K., Preziosi, R.F., Mating system variation in neotropical black mangrove, *Avicennia germinans*, at three spatial scales towards an expanding northern distributional limit, *Estuarine, Coastal and Shelf Science* (2020), doi: https://doi.org/10.1016/j.ecss.2020.106712.

John Paul Kennedy: Conceptualization, Methodology, Investigation, Supervision, Visualization, Writing – original draft. **Joshua M. Sammy**: Investigation, Writing – review & editing. **Jennifer K. Rowntree**: Funding acquisition, Conceptualization, Supervision, Writing – review & editing. **Richard F. Preziosi**: Funding acquisition, Conceptualization, Supervision, Writing – review & editing.

Mating system variation in neotropical black mangrove, *Avicennia germinans*, at three spatial scales towards an expanding northern distributional limit

John Paul Kennedy*, Joshua M. Sammy, Jennifer K. Rowntree[1], Richard F. Preziosi[1]

Ecology and Environment Research Centre, Department of Natural Sciences, Faculty of Science and Engineering, Manchester Metropolitan University, Manchester, UK

* Corresponding author.

E-mail address: john.p.kennedy@stu.mmu.ac.uk; kennedy3jp@gmail.com (J.P. Kennedy).

[1] Equal contribution as senior author.

Abstract

Climate-driven range expansion of ecosystem-defining foundation species can have wide-reaching ecological consequences. Expansion may also result in mating system changes in these foundation species because of the ecological characteristics of range margins, such as greater conspecific isolation and reduced pollinator availability. It is important to understand how mating systems may change during expansion due to their direct influence on intraspecific genetic and demographic dynamics. Here, we used 12 microsatellite loci to genotype progeny arrays of the neotropical black mangrove (*Avicennia germinans*) at six collection sites (n = 23 maternal trees; 1,612 genotyped propagules) along a latitudinal gradient towards a northern distributional limit on the Atlantic coast of Florida, USA (27.56 – 30.01°N), where mangroves have expanded into salt marsh over the past several decades. We assessed mating system variation at three spatial scales. First, at the species-distribution level, published outcrossing rates for tropical conspecifics were more than two times higher than those for subtropical Florida *A.*

germinans, consistent with reductions in pollinator diversity and in mangrove abundance with latitude. Second, at the population level, Florida outcrossing rates did not systematically decline towards the northern range limit, but instead, a more open pollen-dispersal neighbourhood at the transition from mangrove to salt marsh dominance may elevate outcrossing until conspecific abundances become too low towards the range limit. Third, at the individual level, outcrossing increased as conspecific cover increased at the Florida range margin, consistent with density-dependent plastic shifts in mating system. These findings suggest that ecological structure influences the *A. germinans* mating system at varying spatial scales. Further research needs to evaluate the effect of *A. germinans* mating system variation on the survival and fitness of offspring and on the extent of population-level local adaptation at expanding distributional limits.

Keywords: density-dependent; foundation species; outcrossing; pollinator diversity; range expansion; self-fertilisation

Introduction

Climate-driven redistributions of species are now commonplace and can lead to important changes in ecological communities, ecosystem function, and human well-being (Pecl et al., 2017). For instance, range expansion of ecosystem-defining foundation species (e.g., long-lived tree and shrub species) can result in entire biome shifts with wide-reaching ecological consequences (Beck et al., 2011; Peñuelas and Boada, 2003; Saintilan et al., 2014).

Colonisation of new habitat has long been associated with greater self-fertilisation in plants (i.e., Baker's Law; Baker, 1955) as characteristics of expanding range margins, such as greater

isolation among conspecifics (Eckert et al., 2010; Ghazoul, 2005) and reductions in pollinator availability (Kalisz et al., 2004; Moeller et al., 2012; Yin et al., 2016), are known to select for increased self-fertilisation (Hargreaves and Eckert, 2014). However, adaptive shifts in mating system are not thought to be general attributes of the expansion of long-lived trees and shrubs because these species generally maintain outcrossing independent of their environment (Barrett and Harder, 2017). Instead, density-dependent plastic shifts towards greater self-fertilisation may occur during initial colonisation (Morgan et al., 2005; Peterson and Kay, 2015), with subsequent changes post-colonisation as increased conspecific density favours shifts back towards greater outcrossing (Pannell, 2015). Understanding mating system variation is important because of its direct influence on genetic and demographic dynamics within a species (Barrett and Harder, 2017 and cites within).

Mangroves are an assortment of intertidal tree and shrub species that are ecologically-important coastal foundation species (Tomlinson, 1986). Mangroves originate in the tropics and decline in abundance towards latitudinal range limits that correspond to ecological thresholds in temperature and/or precipitation (Osland et al., 2017). The pantropical genus *Avicennia* consists of eight species, of which three are found in the Neotropics (Duke, 1992). Of these three neotropical *Avicennia* species, *Avicennia germinans* (neotropical black mangrove) is the most widespread, with a distribution across the tropics and into the subtropics (Lonard et al., 2017). On the Atlantic coast of Florida (USA), mangroves are the dominant coastal foundation species at lower latitudes where three mangrove species (*A. germinans*, plus *Rhizophora mangle* and *Laguncularia racemosa*) form dense forests, but the higher-latitude mangrove range margin consists of discrete patches of isolated individuals (almost exclusively *A. germinans*) in a salt marsh-dominated landscape (Kangas and Lugo, 1990). Lack of extreme winter freezes over the

68 past several decades have been linked to proliferation and expansion of *A. germinans* at this
69 northern distributional limit (Cavanaugh et al., 2019, 2014; Rodriguez et al., 2016) and further
70 expansion is forecast with climate change (Cavanaugh et al., 2019, 2015). This ongoing shift
71 from salt marsh to mangrove dominance at this expanding range margin will presumably lead to
72 significant changes in ecosystem structure and services essential to human well-being, including
73 nutrient storage, storm protection, and habitat availability for certain fauna (Doughty et al., 2017,
74 2016; Kelleway et al., 2017; Osland et al., 2018; Simpson et al., 2019).

75 *Avicennia germinans* is hermaphroditic with clusters of white flowers (each with one stigma
76 and four stamens) on axillary or terminal inflorescences (Lonard et al., 2017), and is recognised
77 as predominantly outcrossed via insect pollination (Tomlinson, 1986). However, higher-latitude
78 mangroves may encounter far less diverse sets of pollinators than their tropical conspecifics
79 (Hermansen et al., 2014b). Few pollination studies exist for *A. germinans*, but those that do
80 demonstrate greater pollinator diversity at lower latitudes (Sánchez-Núñez and Mancera-Pineda,
81 2012) compared to higher latitudes (Landry, 2013). Consistent with this latitudinal decline in
82 pollinator diversity, indirect genetic evidence (via inbreeding coefficients, F_{IS}) supports
83 predominant outcrossing in *A. germinans* populations closer to the range centre of this species
84 (Cerón-Souza et al., 2012; Mori et al., 2015; Nettel et al., 2008; Ochoa□Zavala et al., 2019; but,
85 see Salas-Leiva et al., 2009), with observations of elevated inbreeding towards range limits
86 (Kennedy et al., 2020a; Mori et al., 2015; Ochoa□Zavala et al., 2019). Direct evidence via
87 progeny arrays with *A. germinans* in Mexico also supports predominant outcrossing closer to the
88 range centre (Nettel-Hernanz et al., 2013), but we still lack equivalent direct estimates of mating
89 system towards *A. germinans* distributional limits.

Here, we used progeny arrays to estimate *A. germinans* outcrossing rates at six collection sites along a latitudinal gradient towards the expanding northern distributional limit of this species on the Atlantic coast of Florida. We then assessed variation in mating system (i.e., outcrossing versus self-fertilisation rates; Neal and Anderson, 2005) at three spatial scales. First, at the species-distribution level, we compared published outcrossing rates based on progeny arrays from tropical conspecifics to rates observed here for subtropical *A. germinans*. Second, at the population level, we assessed whether outcrossing rates decreased along the Florida latitudinal gradient that reaches the northern range limit of this species. Third, at the individual level, we assessed whether conspecific cover influences outcrossing rates of individual trees at the expanding range margin. We tested the following predictions: (1) outcrossing rates will decline from the tropics to subtropics; (2) population-level outcrossing rates will decline along the latitudinal gradient in Florida; (3) tree-level outcrossing rates will increase as conspecific cover increases at the Florida range margin.

Materials and methods

2.1 Collection sites and sampling methods

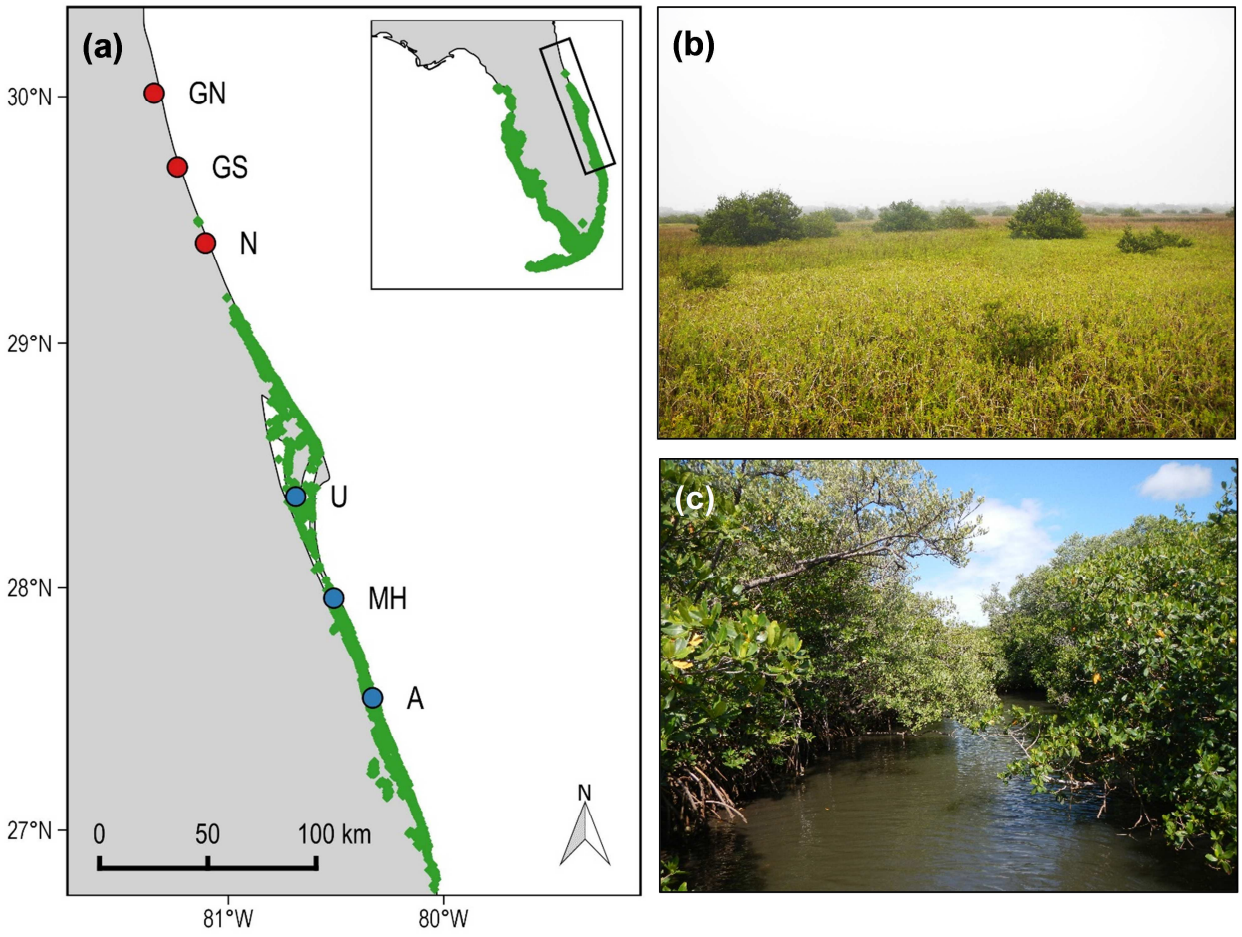
On 7-8 October 2017, we collected ~100 propagules from and recorded GPS coordinates for each of 30 maternal trees at six collection sites (n = 5 trees per site) along a latitudinal gradient (27.56 – 30.01^{o}N) on the Atlantic coast of Florida. We systematically collected propagules from around the entire canopy of each maternal tree. A leaf was also collected from each maternal tree and dehydrated in silica gel to obtain maternal genotypes. Atlantic Florida mangroves are replaced by salt marsh as the dominant coastal foundation species at approximately 29 ^{o}N (Spalding et al., 2010). Our three most southern collection sites (27.56 – 28.37^{o}N) are areas

within the *A. germinans* continuous range core where mangroves are the dominant coastal foundation species; whereas, our three most northern collection sites (29.41 – 30.01^{o}N) are areas at the *A. germinans* range margin where salt marsh species are dominant and *A. germinans* exists as discrete patches of isolated individuals (Fig. 1). Population genetic data for each of these collection sites was previously collected in 2015 (Table 1; Kennedy et al., 2020a). All propagules from an individual tree were kept together in one plastic bag during field collections. Propagules that were eventually genotyped had their pericarps removed and were stored at -20^{o}C until DNA extraction.

Our study design reflects two principal factors: (1) Atlantic Florida *A. germinans* exhibits a considerable reduction in genetic variation towards the northern distributional limit (Kennedy et al., 2020a) and (2) limited polymorphism can restrict our ability to detect differences using molecular markers (Arnaud-Haond et al., 2005). We were concerned that reduced genetic variation may inhibit our ability to quantify reliable outcrossing rates and, as such, we needed to focus more sampling effort on the number of offspring per tree. This concern proved valid, as we determined that a relatively large number of offspring were needed to obtain reliable tree-level outcrossing estimates (see *2.3 Descriptive analyses*). Hence, for practical reasons, we were limited in the number of sampled maternal trees that could be included in this study. We included 23 maternal trees. From south to north, we genotyped progeny arrays from n = 5, 2, and 2 maternal trees at each of the three range-core sites, and n = 4, 5, and 5 maternal trees at each of the three range-margin sites (Table 1). We focussed more effort on the range-margin sites to better address our third prediction (i.e., tree-level outcrossing rates will increase as conspecific cover increases at the range margin). Although only two maternal trees were analysed at two of the range-core sites, we attempted to capture variation across as large of an area as possible.

136 These two forest patches (code: MH, U; Table 1) extend for approximately 2.3 and 3.9 km,
137 respectively, along the adjacent river channel, and we selected trees that were separated by 1.3
138 and 1.2 km, respectively (i.e., 56% and 31% of the linear extent of these collection sites).

139



140

141 **Fig. 1.** Six collection sites along a latitudinal gradient (27.56 – 30.01°N) on the Atlantic coast of
142 Florida (USA) for *Avicennia germinans* progeny arrays. (a) The three most southern sites (blue
143 circles) are areas within the continuous range core of this species, where mangroves are the
144 dominant coastal foundation species. The three most northern collection sites (red circles) are

areas at the range margin of this species, where salt marsh species are dominant and *A. germinans* exists in discrete patches. Mangrove distribution is shown in green (Giri et al., 2011). (b, c) Representative picture of range-margin and range-core community structure, respectively.

2.2 DNA isolation and microsatellite genotyping

For leaves from the 23 maternal trees, genomic DNA was isolated from 20 mg of dry tissue with the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) following the standard protocol, with an extended incubation of 45 minutes. Trees were genotyped at 12 previously-developed nuclear microsatellite loci (Cerón-Souza et al., 2012, 2006; Mori et al., 2010; Nettel et al., 2005) following the protocol outlined in Kennedy et al. (2020a). We performed PCR on a Prime thermal cycler (Techne, Straffordshire, UK), analysed fragments on an Applied Biosystems 3730 DNA Analyzer (Applied Biosystems, Foster City, California, USA) with LIZ 500 size standard, and scored alleles in the R-package Fragman (Covarrubias-Pazaran et al., 2016). We amplified and genotyped DNA from each maternal tree twice to ensure we had the correct multi-locus genotype.

For each propagule, we removed the cotyledons and extracted DNA from portions of the hypocotyl and radicle, the eventual stem and root of the germinating seedling. Genomic DNA from propagules was isolated from 50 mg of frozen hypocotyl/radicle tissue with the DNeasy 96 Plant Kit (Qiagen, Hilden, Germany) following the standard protocol. Subsequent genotyping steps were identical to those for leaves, but we modified the PCR volumes outlined in Kennedy et al. (2020a). Each of the two multiplex PCR contained a total volume of 6 μL with 2.5 μL Multiplex PCR Master Mix, 0.5 μL primer mix, and 3 μL of genomic DNA. We also randomly

167 re-amplified and re-genotyped 5% of our propagule DNA samples to estimate a study error rate
168 (Bonin et al., 2004).

169

170 *2.3 Descriptive analyses: maternal genotypes and sample sizes*

171 We performed a discriminant analysis of principal components (DAPC) (Jombart et al., 2010) in
172 the R-package adegenet 2.1.1 (Jombart and Ahmed, 2011) as a visual assessment of genetic
173 differences among the 23 maternal trees. We retained nine principal components, the minimum
174 number that explained ~90% of the total variance, identified two clusters, and retained three
175 discriminant functions. We extracted each individual's coordinates on the two principal axes of
176 the DAPC (i.e., ind.coord) and plotted them in ggplot2 (Wickham, 2011).

177 We performed an initial analysis to estimate how many genotyped propagules would be
178 needed to provide reliable tree-level outcrossing estimates. For each of two trees (code: MH1,
179 MH3), we genotyped as close to 100 propagules as possible (n = 87, 82, respectively) and
180 estimated tree-level detectable outcrossing rates with the direct approach outlined below (see *2.4*
181 *Outcrossing calculations*). We then reduced the number of propagules in the data set by
182 increments of five (i.e., n = 80, 75, 70, 65, 60, 55, 50, 45) and re-calculated outcrossing rates for
183 each of these new subsets. This approach enabled us to visualise how outcrossing estimates
184 changed as sample sizes decreased. Estimates of these subsets remained relatively consistent
185 with the initial estimates (MH1: 0.6 – 6.8% change; MH3: 2.5 – 9.3% change) until $n \leq 55$ when
186 values became more variable (MH1: 7.7 – 13% change; MH3: 16.1 – 43.1% change) (Fig. 2). As
187 such, we determined that $n \geq 60$ propagules per tree should be sufficient to estimate reliable tree-
188 level outcrossing rates.

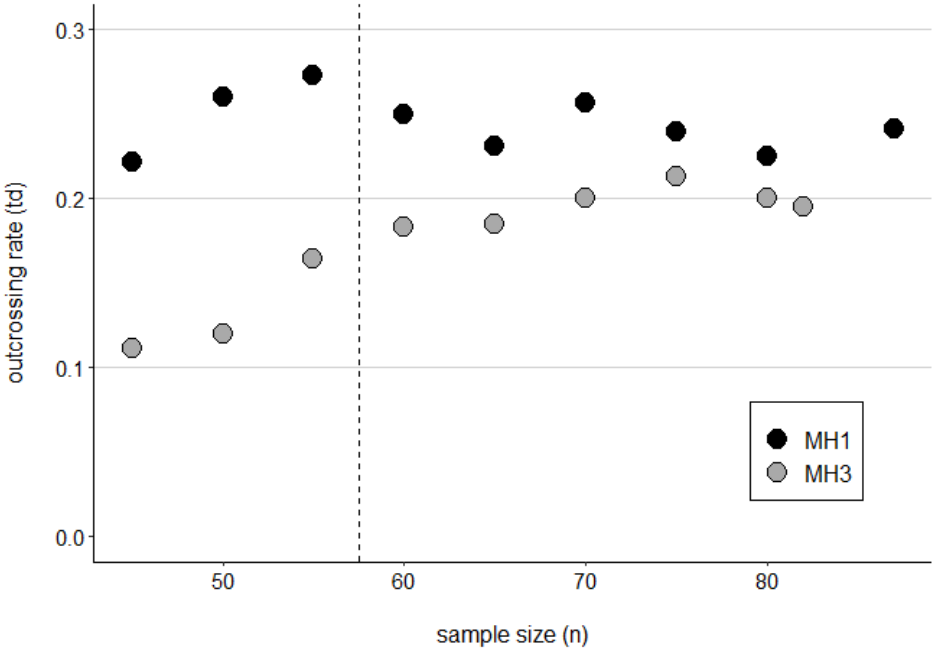


Fig. 2. Initial analysis to estimate an appropriate sample size of propagules to calculate tree-level outcrossing rates. Systematic reductions in sample size for two trees (MH1, MH3) continued to provide estimates of detectable outcrossing rate (t_d) relatively consistent with initial estimates until $n \leq 55$ when values became more variable (MH1: 7.7 – 13% change; MH3: 16.1 – 43.1% change). This threshold is shown with a vertical dashed line. We determined that $n \geq 60$ propagules should provide reliable estimates of tree-level outcrossing rates.

2.4 Outcrossing calculations: population-level and tree-level

To assess population-level mating system variation towards the Florida *A. germinans* range limit, we calculated multi-locus outcrossing rates (t_m) for each of the six collection sites with the maximum likelihood-based MLTR (Ritland, 2002). We also calculated levels of biparental inbreeding ($t_m - t_s$) and we used the proportion of offspring with the same father [$r_p(m)$] to

calculate the number of effective pollen donors [1 / r_p(m)] for each collection site. We used default parameters, 500 bootstraps to calculate standard errors, and resampled among individuals within families.

To assess mating system variation among individual trees, we used a direct approach based on allelic differences between maternal genotypes and their sampled offspring to calculate apparent outcrossing rates (Cruzan et al., 1994). First, detectable outcrossing rates (t_d) were calculated as the number of propagules that possessed an allele not present in the maternal genotype (i.e., detectable outcross event) divided by the number of propagules genotyped. However, there may also be a percentage of offspring that appear to be the product of self-fertilisation, but are instead the result of outcrossing to individuals with similar genotypes to the maternal tree (i.e., undetectable outcross event). Hence, maternal genotypes with high-frequency alleles in a population will result in greater undetected outcrossing. Using allele frequency data from each of these six collection sites in 2015 (Kennedy et al., 2020b), we calculated the probability of an undetectable outcross event [$P(u)_j$] for each of the 23 maternal trees as the product across loci of the frequencies of each maternal allele within the corresponding collection site (Cruzan et al., 1994). For each maternal tree, we multiplied its specific $P(u)_j$ by the number of propagules that were not identified as outcrossed to obtain an estimate of the number of potential undetected outcross events. We then calculated apparent outcrossing rates (t_a) as the number of detectable outcross events plus the number of potential undetected outcross events divided by the number of propagules genotyped. We also estimated these tree-level outcrossing rates with the maximum likelihood-based MLTR, as described above for population-level estimates.

226 *2.5 Estimates of conspecific cover at the range margin*

227 It remains difficult to remotely assess mangrove cover at fragmented range margins (Bunting et
228 al., 2018). However, mangrove patches within salt marsh can generally be manually identified
229 with relative ease in satellite images, while also aided by knowledge of the collection site. For
230 each of the three range-margin collection sites, we manually measured approximate estimates of
231 area covered with *A. germinans* within a 10 m radius circle around each maternal tree (n = 14
232 GPS-referenced trees) with the polygon function in Google Earth Pro 7.3.2.5776. We chose this
233 size because 10 m was the longest distance that permitted reliable estimates around maternal
234 trees adjacent to terrestrial hammock forest patches.

235

236 *2.6 Statistical analyses*

237 We performed all statistical analyses in R v3.4.2 (R Core Team, 2013). To test prediction 1
238 (decline in outcrossing from tropics to subtropics), we compared mean multi-locus outcrossing
239 rates (t_m) for these Florida collection sites (n = 6 sites) to published values for conspecifics in
240 Mexico (n = 3 sites; Nettel-Hernanz et al., 2013) with a two-sample t-test. To test prediction 2
241 (decline in outcrossing along the Florida latitudinal gradient), we evaluated the relationship
242 between t_m and latitude with a Spearman's rank correlation (n = 6 sites). To test prediction 3
243 (increase in outcrossing with increased conspecific cover at the range margin), we evaluated the
244 effect of *A. germinans* cover around each range-margin maternal tree on tree-level apparent
245 outcrossing rates (t_a) with a linear regression (n = 14 trees). We natural log-transformed *A.*
246 *germinans* cover to meet the statistical assumption of normality.

247

Results

3.1 Maternal genotypes, sample sizes and study error rate

Each of the 23 maternal trees produced consistent multi-locus genotypes after being amplified and genotyped a second time. All 23 maternal genotypes were unique, with a range from 1 of 12 to 10 of 12 matching loci, and exhibited a clear separation between range core and margin genotypes (Fig. 3). We genotyped a total of 1,612 propagules, with a mean sample size per tree of 70.1 ± 5.3 (SD; range: 64 – 87). We re-amplified and re-genotyped DNA from 87 propagules (5.4% of all samples) and found an error rate of 0.01% (one error out of 1,044 locus comparisons). This locus-specific error was removed from the data set.

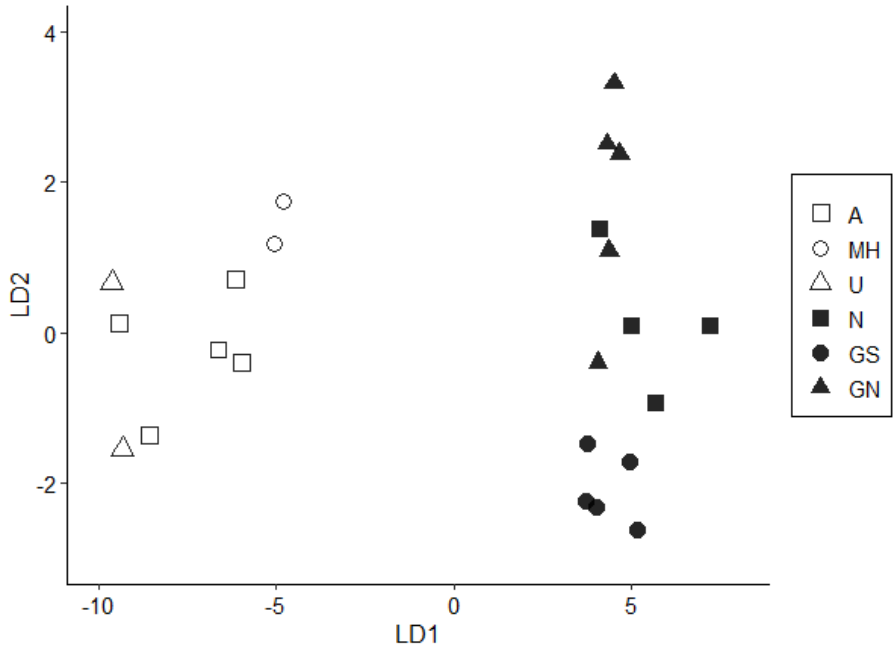


Fig. 3. Discriminant analysis of principal components (DAPC) of maternal tree multi-locus genotypes. All 23 maternal trees possessed unique multi-locus genotypes and exhibited a clear separation between range core and margin. Open shapes indicate range-core sites (A, Avalon;

MH, Maritime Hammock; U, Ulumay); filled shapes indicate range-margin sites (N, North Peninsula; GS, GTM South; GN, GTM North).

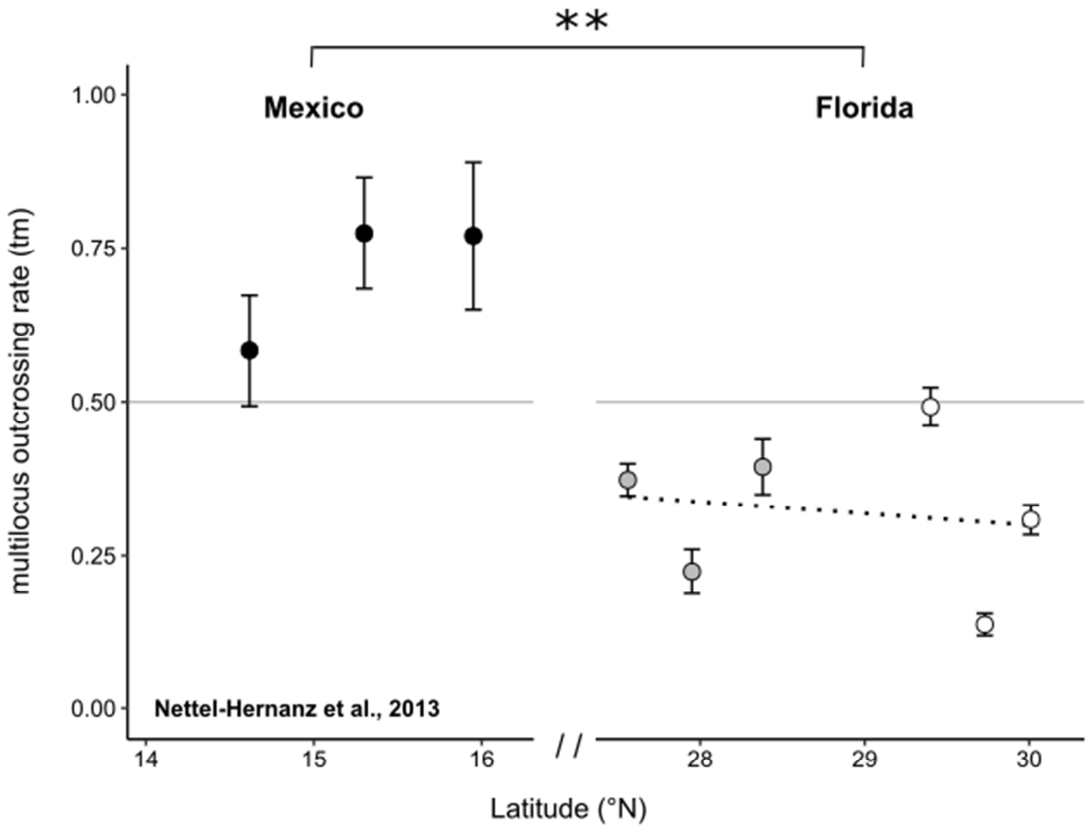
3.2 Population-level mating system variation

Population-level multi-locus outcrossing rates (t_m) in Florida ranged from 0.14 ± 0.02 (SE) to 0.49 ± 0.03 (Table 1). Florida t_m were, on average, more than two times lower than published values for tropical conspecifics in Mexico (mean t_m Florida: 0.32, Mexico: 0.71; $t_{4.8} = -4.8$, $p = 0.006$) (Fig. 4).

Population-level outcrossing (t_m) did not systematically decline along the Florida latitudinal gradient (Spearman's correlation, $r_s = -0.20$, $p = 0.70$). Instead, highest and lowest values were observed in range-margin sites (Fig. 4). All collection sites exhibited low levels of biparental inbreeding ($t_m - t_s = -0.01 - 0.06$) (Table 1). MLTR could not calculate the proportion of offspring with the same father [$r_p(m)$] for one range-margin site (code: GS), presumably because this site exhibited the lowest outcrossing rates (Table 1, 2). Estimates of $r_p(m)$ were calculated for all other collection sites and the number of effective pollen donors [$1 / r_p(m)$] varied from 2.5 to 20.4, with the lowest number of pollen donors at the northern range limit (code: GN) and the highest number at the most southern range-margin site (code: N) (Table 1).

Table 1 Population-level genetic diversity and mating system parameters of *Avicennia germinans* from six collection sites on the Atlantic coast of Florida (USA). AR, allelic richness; H_S, unbiased gene diversity; arrays, number of progeny arrays sampled; n, total number of propagules genotyped; t_m, multi-locus outcrossing rate; $t_m - t_s$, biparental inbreeding; $r_p(m)$, proportion of offspring with the same father; $1 / r_p(m)$, number of effective pollen donors. Standard error in parentheses. Note: estimates of $r_p(m)$ for collection site GS could not be calculated. †Data from Kennedy et al. (2020a) based on n = 30 adult trees per collection site genotyped at the same 12 nuclear microsatellite loci used here.

			genetic diversity†				progeny arrays								
Site	Code	Latitude	AR		H_S		arrays	n	t_m		$t_m - t_s$		$r_p(m)$		$1/r_p(m)$
Avalon	A	27.5468	3.40	(0.64)	0.42	(0.05)	5	336	0.37	(0.03)	0.03	(0.01)	0.16	(0.04)	6.2
Maritime Hammock	MH	27.9566	3.14	(0.48)	0.42	(0.05)	2	169	0.22	(0.04)	0.03	(0.01)	0.17	(0.07)	5.8
Ulumay	U	28.3756	2.33	(0.24)	0.31	(0.05)	2	135	0.39	(0.05)	-0.01	(0.02)	0.08	(0.12)	13.2
North Peninsula	N	29.4096	2.83	(0.61)	0.35	(0.06)	4	277	0.49	(0.03)	0.00	(0.02)	0.05	(0.02)	20.4
GTM South	GS	29.7159	2.85	(0.42)	0.40	(0.06)	5	342	0.14	(0.02)	0.02	(0.01)	-	-	-
GTM North	GN	30.0144	2.14	(0.29)	0.21	(0.05)	5	353	0.31	(0.02)	0.06	(0.01)	0.41	(0.07)	2.5



286

287 **Fig. 4.** Population-level multi-locus outcrossing rates (t_m) for tropical *Avicennia germinans* in
288 Mexico (Nettel-Hernanz et al., 2013) and subtropical *A. germinans* in Florida (this study).
289 Florida *A. germinans* mean t_m was more than two times smaller than their tropical conspecifics
290 ($t_{4.8}$ = -4.8, p = 0.006). Outcrossing (t_m) did not systematically decline along the Florida
291 latitudinal gradient (r_s = -0.20, p = 0.70). Non-significant correlation is depicted with a dotted
292 line. *A. germinans* in Mexico is shown in black, in Florida range core is shown in grey, and in
293 Florida range margin is shown in white. Population error bars are standard error. **, $p < 0.01$.

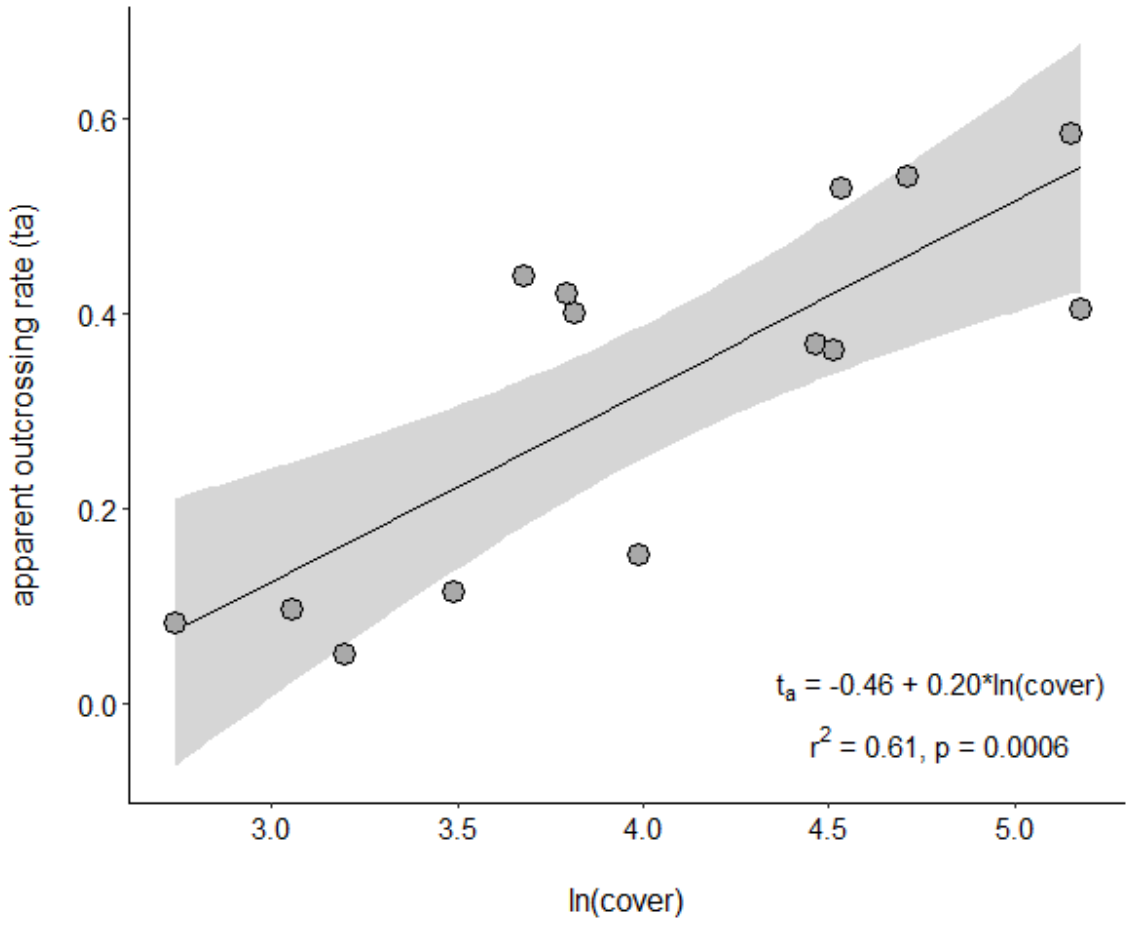
294 *3.3 Tree-level mating system variation*

295 Tree-level detectable outcrossing rates (t_d) ranged from 0.05 to 0.58 (Table 2). Probabilities of an
296 undetectable outcross event were generally low (0 – 0.09) and, as such, the number of potential
297 undetected outcross events was also generally low (0 – 4.7) (Table 2). However, there were two
298 notable exceptions with probabilities of 0.17 and 0.22, and a total of 9 and 12 potential
299 undetected outcross events (tree: GN1, GN4; Table 2). These two exceptions were trees at the
300 northern range limit, the least genetically-diverse collection site (Table 1). After incorporating
301 potential undetected outcross events into our calculations, tree-level apparent outcrossing rates
302 (t_a) still ranged from 0.05 to 0.58 (Table 2) and were highly correlated with t_d (Pearson's
303 correlation, $r = 0.97$, $p < 0.0001$). Tree-level estimates calculated with MLTR were also highly
304 correlated with both t_d ($r = 0.99$, $p < 0.0001$) and t_a ($r = 0.97$, $p < 0.0001$).

305 Conspecific cover at the range margin, measured as the area covered by *A. germinans* within
306 a 10 m radius circle around each range-margin maternal tree, varied considerably from 15.5 to
307 177.4 m^2 (7 – 57% cover), with highest values at the most southern range-margin site (tree: N2-
308 5; Table 2). Apparent outcrossing rates (t_a) increased as conspecific cover increased ($t_a = -0.46 +$
309 $0.20*\ln(\text{cover})$, $F_{1,12} = 21.0$, $p = 0.0006$, $r^2_{adj} = 0.61$), with a 2% increase in outcrossing with
310 every 10% increase in *A. germinans* cover (m^2) (Fig. 5).

311 **Table 2** Tree-level outcrossing rates and conspecific cover around range-margin trees. n, number
312 of propagules genotyped; t_d, detectable outcrossing rate; $P(u)_j$, probability of an undetectable
313 outcross event; undetected, number of potential undetected outcross events; t_a, apparent
314 outcrossing rate; cover, *A. germinans* cover within a 10 m radius circle around each maternal tree
315 at range-margin sites. Note: cover was not calculated for range-core collection sites.
316

Tree	Latitude	Longitude	n	t_d	$P(u)_j$	undetected	t_a	cover
A1	27.560644	-80.328730	72	0.33	0.000	0.0	0.33	-
A2	27.559835	-80.329391	67	0.49	0.050	1.7	0.52	-
A3	27.559641	-80.329857	66	0.42	0.002	0.1	0.43	-
A4	27.559870	-80.330614	64	0.17	0.003	0.2	0.17	-
A5	27.557966	-80.329410	67	0.42	0.014	0.6	0.43	-
MH1	27.958072	-80.515093	87	0.24	0.004	0.3	0.24	-
MH3	27.951081	-80.509048	82	0.20	0.001	0.1	0.20	-
U3	28.372275	-80.684404	70	0.23	0.088	4.7	0.30	-
U5	28.380151	-80.685456	65	0.52	0.003	0.1	0.52	-
N2	29.407971	-81.099778	65	0.40	0.006	0.2	0.40	177.4
N3	29.407679	-81.098927	70	0.40	0.035	1.5	0.42	44.5
N4	29.407872	-81.099167	70	0.53	0.026	0.9	0.54	111.1
N5	29.407941	-81.099617	72	0.58	0.002	0.1	0.58	173.2
GS1	29.729168	-81.240662	65	0.05	0.005	0.3	0.05	28.2
GS2	29.730158	-81.240638	66	0.06	0.038	2.3	0.10	21.3
GS3	29.730077	-81.241591	70	0.10	0.016	1.0	0.11	32.9
GS4	29.730190	-81.241972	71	0.14	0.013	0.8	0.15	54.1
GS5	29.729601	-81.242441	70	0.31	0.079	3.8	0.37	87.1
GN1	30.016524	-81.345922	70	0.23	0.222	12.0	0.40	45.5
GN2	30.016660	-81.345954	72	0.43	0.014	0.6	0.44	39.7
GN3	30.014781	-81.344683	72	0.49	0.081	3.0	0.53	93.2
GN4	30.014178	-81.344754	69	0.23	0.171	9.0	0.36	91.2
GN5	30.013645	-81.345022	70	0.06	0.026	1.7	0.08	15.5



317

318 **Fig. 5.** Tree-level apparent outcrossing rates (t_a) increased as conspecific cover [ln(cover)]
319 increased at the *A. germinans* range margin on the Atlantic coast of Florida (USA). Conspecific
320 cover was measured as the area covered by *A. germinans* within a 10 m radius circle around each
321 maternal tree at the three range-margin collection sites.

322

323 **Discussion**

324 We used progeny arrays to characterise mating system in *Avicennia germinans* at six collection
325 sites along a latitudinal gradient towards the northern distributional limit of this species on the
326 Atlantic coast of Florida (27.56 – 30.01°N). We assessed variation in mating system at three

spatial scales: (1) at the species-distribution level, (2) at the population level along the Florida latitudinal gradient, and (3) at the individual level among conspecifics at the expanding Florida range margin. First, published outcrossing rates for tropical conspecifics in Mexico were, on average, more than two times higher than those for subtropical Florida *A. germinans*. Second, population-level outcrossing rates did not systematically decline with latitude towards the northern range limit in Florida. Third, tree-level outcrossing rates increased as conspecific cover increased at the Florida range margin.

4.1 Reduced outcrossing from tropics to subtropics

In contrast to the prevailing idea that *A. germinans* is predominantly outcrossed, we found that all Florida collection sites were predominantly self-fertilised. Tropical conspecifics in Mexico (14.61 – 15.95^{o}N) exhibit predominant outcrossing (Nettel-Hernanz et al., 2013), with outcrossing rates more than double our observations for subtropical Florida *A. germinans*. Predominant outcrossing has also been documented for a co-occurring neotropical congener, *A. schaueriana*, at lower latitude (0.82^{o}S) (Mori et al., 2015). Reduced outcrossing towards *A. germinans* distributional limits is consistent with a global decline in plant outcrossing rates towards higher latitude (Moeller et al., 2017). However, Moeller et al. (2017) found that this global pattern is mostly shaped by latitudinal changes in life history and growth form (i.e., lower latitude: greater frequency of perennial and tree species; higher latitude: greater frequency of annual and herbaceous species), and propose that evaluations of mating system variation in species with broad latitudinal distributions will help better understand the potential impact of plant-pollinator interactions in shaping latitudinal patterns in mating systems.

Reduced outcrossing in subtropical *A. germinans* is consistent with reductions in pollinator diversity across this species' broad distribution range. At lower latitude, four highly-effective pollinators were identified on the small, Caribbean island of San Andrés (12.54^{o}N) (Sánchez-Núñez and Mancera-Pineda, 2012), and a diverse set of pollinators (including wasps, flies, and bees) was also identified for the neotropical congener, *A. schaueriana* (7.68^{o}S) (Nadia et al., 2013). At higher latitude, only one principal pollinator, the exotic European honey bee (*Apis mellifera*), accounted for 87% of flower visits at a location in Southeast Florida (26.03^{o}N) (Landry, 2013). However, we cannot disregard additional factors that could also impact the *A. germinans* mating system in Florida, such as reduced mangrove abundances towards distributional limits (Osland et al., 2017) and the highly-fragmented nature of Atlantic Florida mangroves due to anthropogenic modifications of these coastal ecosystems (Brockmeyer et al., 1996). Outcrossing often increases with conspecific density and pollinator abundance, but these two factors are not independent for animal-pollinated plants because dense groupings generally attract more pollinators (Ghazoul, 2005). Research near the southern latitudinal limits (33.94 – 34.63^{o}S) of a widespread Indo-West Pacific congener, *A. marina*, highlights this point. Exotic European honey bees were also identified as the only significant pollinator for this species (Hermansen et al., 2014b) and smaller, more fragmented stands attracted fewer pollinators with altered foraging behaviour that may facilitate greater self-fertilisation (i.e., bees spent more time foraging on individual trees) (Hermansen et al., 2014a). As a result, these smaller *A. marina* stands exhibited reduced outcrossing compared to larger stands (Hermansen et al., 2015). Our observation of reduced outcrossing in subtropical Florida *A. germinans* may be the product of the interactive effects of reduced pollinator diversity and more fragmented mangrove area, that could also reduce pollinator abundances, compared to tropical mangrove forests. Further research

372 needs to directly link assessments of mating system with plant-pollinator surveys across the
373 broad, latitudinal distribution of *A. germinans*, as well as other *Avicennia* species, to provide
374 definitive answers. In addition, research also needs to consider further environmental (e.g.,
375 seasonality; Chybicki and Dzialuk, 2014; Yin et al., 2016) and biological factors (e.g., fecundity,
376 phenology; Ghazoul, 2005; Kameyama and Kudo, 2015) that can shape mating system variation.

377 Reductions in outcrossing are the product of a combination of biparental inbreeding and
378 self-fertilisation. We found low biparental inbreeding at all collection sites, consistent with *A.*
379 *germinans* progeny arrays in Mexico ($t_m - t_s = 0.01 - 0.06$; Nettel-Hernanz et al., 2013).
380 However, progeny arrays with the neotropical congener, *A. schaueriana*, exhibited a greater
381 effect of biparental inbreeding ($t_m - t_s = 0.15$; Mori et al., 2015), and even higher rates were
382 found at the southern latitudinal limits of the Indo-West Pacific congener, *A. marina* ($t_m - t_s =$
383 0.29 – 0.53; Hermansen et al., 2015). Differences among these studies may be explained by
384 interspecific variation in reproductive biology, variation in kinship structure among collection
385 sites (Hasan et al., 2018), or simply methodological differences (e.g., number of progeny arrays,
386 of propagules sampled, and of loci genotyped) that can impact these parameter estimates
387 (Ritland, 2002). Our findings suggest that reduced outcrossing in subtropical Florida *A.*
388 *germinans* is predominantly via self-fertilisation, either autonomous (within the same flower) or
389 geitonogamous (among flowers on the same plant). Although we lack evidence for *A. germinans*,
390 multiple *Avicennia* species are self-compatible, including the neotropical congener, *A.*
391 *schaueriana* (Nadia et al., 2013), and three Indo-West Pacific congeners (Aluri, 1990; Raju et al.,
392 2012), with a much higher fruit set via geitonogamous compared to autonomous self-fertilisation
393 for all species. Geitonogamous self-fertilisation is common in bee-pollinated plants (Harder and
394 Barrett, 1995; Mitchell et al., 2004), is thought to aid mangrove colonisation (Primack et al.,

1981), and seems to be a likely mechanism in *A. germinans* as inflorescences have multiple flowers open simultaneously.

4.2 Density-dependent mating system variation towards range limits

We did not observe a systematic decline in outcrossing rates towards the expanding *A. germinans* northern range limit in Florida, consistent with the prevailing view that adaptive shifts in mating system are not common during range expansion of long-lived trees and shrubs (Barrett and Harder, 2017). Instead, density-dependent plastic shifts in mating system may occur, with transitions between increased self-fertilisation at low density and mixed mating at higher density (Morgan et al., 2005; Peterson and Kay, 2015). We found lowest outcrossing at the range margin, but we also observed highest outcrossing at the most southern range-margin site (Fig. 4). While low density and greater spatial isolation among conspecifics at range margins can reduce outcrossing (Ghazoul, 2005), the same can also be true in areas with higher species diversity that may increase competition for pollinators (Vamosi et al., 2006). Higher-density forests can also restrict routes of pollen vectors and lead to declines in the diversity of tree-level pollen donors (González-Varo et al., 2009). Lower-latitude Florida mangroves consist of dense forests with *A. germinans* and two other mangrove species (*Rhizophora mangle*, *Laguncularia racemosa*), with evidence of competition for pollinators between *A. germinans* and *L. racemosa* (Landry, 2013). In contrast, higher-latitude range-margin Florida mangroves are patches of almost exclusively *A. germinans* individuals surrounded by lower-stature salt marsh species (Kangas and Lugo, 1990). A more open pollen-dispersal neighbourhood and limited interspecific competition at the higher-latitude range margin could facilitate inter-tree pollination among *A. germinans*, consistent with our observation of highest outcrossing and largest number of effective pollen donors at the most

418 southern range-margin site. However, this greater potential for inter-tree pollination would be
419 overshadowed farther north where limited numbers of conspecifics would provide very few
420 potential pollen sources, as evidenced by the lowest number of effective pollen donors at the
421 northern range limit.

422 At the range margin, tree-level outcrossing increased as conspecific cover increased. Lowest
423 outcrossing (t_a = 0.05) at an *A. germinans* cover of 28.2 m^2 (7% of surrounding area covered by
424 *A. germinans*) was more than ten times smaller than highest outcrossing (t_a = 0.58) at a cover of
425 173.2 m^2 (57% covered by *A. germinans*). We presume that continued proliferation of *A.*
426 *germinans* at this range margin, forecast with climate change (Cavanaugh et al., 2019, 2015),
427 will drive further mating system changes as increased conspecific density shifts mating systems
428 towards greater outcrossing (Pannell, 2015). As *A. germinans* becomes more abundant, inter-
429 individual distances will decline, presumably these denser patches will attract more pollinators,
430 and tree-level outcrossing will increase. Hence, documented loss of genetic diversity towards *A.*
431 *germinans* range limits (Kennedy et al., 2020a; Mori et al., 2015; Ochoa□Zavala et al., 2020,
432 2019; Sandoval-Castro et al., 2014), and for other *Avicennia* species (Arnaud-Haond et al., 2006;
433 Binks et al., 2019; De Ryck et al., 2016; Maguire et al., 2000), may be the product of founder
434 effects combined with density-dependent plastic shifts towards greater self-fertilisation. Areas
435 that experience subsequent proliferation and immigration will then transition towards greater
436 outcrossing and increased offspring genetic diversity, but will exhibit unique genetic signatures
437 because of the disproportionate contribution of initial colonisers to the gene pool (e.g., Goldberg
438 and Heine, 2017).

439 Plastic shifts towards greater self-fertilisation at range margins can be advantageous as a
440 form of reproductive assurance (Hargreaves and Eckert, 2014) and tend to elevate seed

441 production when pollinators or mates are unreliable (Morgan et al., 2005). However, these
442 advantages may be offset by the genetic costs associated with inbreeding depression (e.g.,
443 reduced offspring survival and fertility) (Charlesworth and Willis, 2009). These costs are
444 documented near the southern latitudinal limits of the Indo-West Pacific congener, *A. marina*,
445 where smaller, less-outcrossed stands exhibited reduced reproductive success, reduced propagule
446 size, and reduced seedling recruitment compared to larger stands (Hermansen et al., 2017).
447 However, our finding of predominant self-fertilisation in *A. germinans* runs contrary to
448 mangrove performance at the Atlantic Florida range margin. These mangroves have undergone
449 proliferation and expansion for several decades (Cavanaugh et al., 2019, 2014; Rodriguez et al.,
450 2016), with evidence from a co-occurring mangrove species, *Rhizophora mangle*, of precocious
451 reproduction and increased propagule size (Dangremond and Feller, 2016) and greater
452 reproductive success (Goldberg and Heine, 2017) compared to Florida conspecifics farther south.
453 Characterisation of *A. germinans* mating system coupled with assessments of offspring
454 performance at this range margin is needed to garner insights into the potential influence of
455 mating system on these expanding populations.

456

457 *4.3 Considerations*

458 Mating system assessments are a balance between the number of progeny arrays and the number
459 of offspring genotyped per progeny array. Here, we focussed our efforts more on genotyping
460 larger numbers of offspring per progeny array. Our sampling design was shaped by our concern
461 that reduced genetic variation towards this northern distributional limit could inhibit our ability
462 to quantify outcrossing rates. This concern was valid as we found that a substantial tree-level
463 effort ($n \geq 60$ propagules per tree) was likely needed to obtain reliable estimates. In addition,

464 although the probability of an undetectable outcross event was low across most maternal trees,
465 two trees at the northern range limit exhibited relatively high probabilities, with 9 and 12
466 potential undetected outcross events. Estimates based on smaller sample sizes per tree,
467 comparable to research in the tropics (14-18 propagules on average per tree; Nettel-Hernanz et
468 al., 2013), could have been overwhelmed by undetectable outcross events and potentially
469 underestimated outcrossing at this northern range limit.

470 However, a greater investment of resources at the tree-level inevitably limited our ability to
471 genotype progeny arrays from a larger number of maternal trees (n = 2-5 trees per collection
472 site). Our sampling design may have provided robust tree-level estimates, but our estimates
473 scaling up to the population level should be interpreted with caution. Sampling few maternal
474 trees can bias these estimates towards tree-specific outcrossing rates that may not be
475 representative of the entire collection site. Although our estimates (based on n = 23 maternal
476 trees) are consistent with a substantial reduction in outcrossing compared to estimates from
477 tropical conspecifics (based on n = 22 maternal trees; Nettel-Hernanz et al., 2013), and are not
478 consistent with a systematic adaptive shift in mating system towards the northern range limit,
479 further supported by evidence of density-dependent variation at the range margin, more intensive
480 sampling of progeny arrays at each collection site is needed to obtain more definitive estimates
481 of population-level mating system variation. Further research into mating systems at range
482 margins, or areas with reduced genetic variation, should consider an investment in both more
483 progeny arrays and large numbers of offspring per progeny array to generate reliable population-
484 level outcrossing estimates.

485

486 *4.4 Conclusions*

This research suggests that ecological structure influences the mating system of the neotropical black mangrove, *A. germinans*, at varying spatial scales towards its expanding northern distributional limit on the Atlantic coast of Florida. First, subtropical Florida *A. germinans* exhibited significant reductions in outcrossing compared to tropical conspecifics, consistent with reductions in pollinator diversity and mangrove abundance with latitude. Second, the transition from mangrove to salt marsh dominance along Atlantic Florida may create a more open pollen-dispersal neighbourhood that is conducive to elevated *A. germinans* outcrossing, until conspecific abundances become too low towards the range limit. Third, greater inter-individual isolation at the range margin resulted in drastic reductions in tree-level outcrossing, consistent with density-dependent plastic shifts in mating system that we presume will continue to shift towards greater outcrossing as these mangroves continue to proliferate with forecast climate trends. Further research needs to evaluate the effect of *A. germinans* mating system variation on the survival and fitness of offspring and on the extent of population-level local adaptation at expanding distributional limits.

Acknowledgements

This research was funded by a Manchester Metropolitan University studentship to JPK. Many thanks to I Feller and R Feller (and their cast iron skillet) and the Smithsonian Marine Station at Fort Pierce, Florida for logistical support during fieldwork, to W Potdevin for lab assistance, to M Nathan for insights into *A. germinans* pollination systems, and to the University of Manchester Genomic Technologies Core Facility and F Combe for fragment analysis. As always, thank you to A Jara Cavieres, C Kennedy, and M Kennedy for unconditional support and big smiles.

References

Aluri, R.J., 1990. Observations on the floral biology of certain mangroves. Proc. Indian Natl. Sci. Acad. Part B, Biol. Sci. 56, 367–374.

Arnaud-Haond, S., Alberto, F., Teixeira, S., Procaccini, G., Serrão, E.A., Duarte, C.M., 2005. Assessing genetic diversity in clonal organisms: Low diversity or low resolution? Combining power and cost efficiency in selecting markers. J. Hered. 96, 434–440. https://doi.org/10.1093/jhered/esi043

Arnaud-Haond, S., Teixeira, S., Massa, S.I., Billot, C., Saenger, P., Coupland, G., Duarte, C.M., Serrão, E.A., 2006. Genetic structure at range edge: Low diversity and high inbreeding in Southeast Asian mangrove (Avicennia marina) populations. Mol. Ecol. 15, 3515–3525. https://doi.org/10.1111/j.1365-294X.2006.02997.x

Baker, H.G., 1955. Self□compatibility and establishment after ‘“long□distance” dispersal. Evolution (N. Y). 9, 347–349.

Barrett, S.C.H., Harder, L.D., 2017. The Ecology of Mating and Its Evolutionary Consequences in Seed Plants. Annu. Rev. Ecol. Evol. Syst. 48, 135–157. https://doi.org/10.1146/annurev-ecolsys-110316-023021

Beck, P.S.A., Juday, G.P., Alix, C., Barber, V.A., Winslow, S.E., Sousa, E.E., Heiser, P., Herriges, J.D., Goetz, S.J., 2011. Changes in forest productivity across Alaska consistent with biome shift. Ecol. Lett. 14, 373–379. https://doi.org/10.1111/j.1461-0248.2011.01598.x

Binks, R.M., Byrne, M., McMahon, K., Pitt, G., Murray, K., Evans, R.D., 2019. Habitat discontinuities form strong barriers to gene flow among mangrove populations, despite the capacity for long-distance dispersal. Divers. Distrib. 25, 298–309. https://doi.org/10.1111/ddi.12851

Bonin, A., Bellemain, E., Eidesen, P.B., Pompanon, F., Brochmann, C., Taberlet, P., 2004. How to track and assess genotyping errors in population genetics studies. Mol. Ecol. 13, 3261–3273. https://doi.org/10.1111/j.1365-294X.2004.02346.x

Brockmeyer, R.E., Rey, J.R., Virnstein, R.W., Gilmore, R.G., Earnest, L., 1996. Rehabilitation of impounded estuarine wetlands by hydrologic reconnection to the Indian River Lagoon, Florida (USA). Wetl. Ecol. Manag. 4, 93–109. https://doi.org/10.1007/BF01876231

Bunting, P., Rosenqvist, A., Lucas, R.M., Rebelo, L.M., Hilarides, L., Thomas, N., Hardy, A., Itoh, T., Shimada, M., Finlayson, C.M., 2018. The Global Mangrove Watch - A New 2010 Global Baseline of Mangrove Extent. Remote Sens. 10, 1669. https://doi.org/10.3390/rs10101669

Cavanaugh, K.C., Dangremond, E.M., Doughty, C.L., Williams, A.P., Parker, J.D., Hayes, M.A., Rodriguez, W., Feller, I.C., 2019. Climate-driven regime shifts in a mangrove–salt marsh ecotone over the past 250 years. Proc. Natl. Acad. Sci. 116, 21602–21608. https://doi.org/10.1073/pnas.1902181116

Cavanaugh, K.C., Kellner, J.R., Forde, A.J., Gruner, D.S., Parker, J.D., Rodriguez, W., Feller, I.C., 2014. Poleward expansion of mangroves is a threshold response to decreased frequency of extreme cold events. Proc. Natl. Acad. Sci. 111, 723–727. https://doi.org/10.1073/pnas.1315800111

Cavanaugh, K.C., Parker, J.D., Cook-Patton, S.C., Feller, I.C., Williams, A.P., Kellner, J.R.,

2015. Integrating physiological threshold experiments with climate modeling to project mangrove species' range expansion. Glob. Chang. Biol. 21, 1928–1938. https://doi.org/10.1111/gcb.12843

Cerón-Souza, I., Bermingham, E., McMillan, W.O., Jones, F.A., 2012. Comparative genetic structure of two mangrove species in Caribbean and Pacific estuaries of Panama. BMC Evol. Biol. 12, 205. https://doi.org/10.1186/1471-2148-12-205

Cerón-Souza, I., Rivera-Ocasio, E., Funk, S.M., McMillan, W.O., 2006. Development of six microsatellite loci for black mangrove (Avicennia germinans). Mol. Ecol. Notes 6, 692–694. https://doi.org/10.1111/j.1471-8286.2006.01312.x

Charlesworth, D., Willis, J.H., 2009. The genetics of inbreeding depression. Nat. Rev. Genet. 10, 783–796. https://doi.org/10.1038/nrg2664

Chybicki, I.J., Dzialuk, A., 2014. Bayesian approach reveals confounding effects of population size and seasonality on outcrossing rates in a fragmented subalpine conifer. Tree Genet. Genomes 10, 1723–1737. https://doi.org/10.1007/s11295-014-0792-3

Covarrubias-Pazaran, G., Diaz-Garcia, L., Schlautman, B., Salazar, W., Zalapa, J., 2016. Fragman: an R package for fragment analysis. BMC Genet. 17, 1–8. https://doi.org/10.1186/s12863-016-0365-6

Cruzan, M.B., Hamrick, J.L., Arnold, M.L., Bennett, B.D., 1994. Mating system variation in hybridizing irises: Effects of phenology and floral densities on family outcrossing rates. Heredity (Edinb). 72, 95–105. https://doi.org/10.1038/hdy.1994.12

Dangremond, E.M., Feller, I.C., 2016. Precocious reproduction increases at the leading edge of a mangrove range expansion. Ecol. Evol. 6, 5087–5092. https://doi.org/10.1002/ece3.2270

De Ryck, D.J.R., Koedam, N., Van der Stocken, T., van der Ven, R.M., Adams, J., Triest, L., 2016. Dispersal limitation of the mangrove Avicennia marina at its South African range limit in strong contrast to connectivity in its core East African region. Mar. Ecol. Prog. Ser. 545, 123–134. https://doi.org/10.3354/meps11581

Doughty, C.L., Cavanaugh, K.C., Hall, C.R., Feller, I.C., Chapman, S.K., 2017. Impacts of mangrove encroachment and mosquito impoundment management on coastal protection services. Hydrobiologia 803, 105–120. https://doi.org/10.1007/s10750-017-3225-0

Doughty, C.L., Langley, J.A., Walker, W.S., Feller, I.C., Schaub, R., Chapman, S.K., 2016. Mangrove Range Expansion Rapidly Increases Coastal Wetland Carbon Storage. Estuaries and Coasts 39, 385–396. https://doi.org/10.1007/s12237-015-9993-8

Duke, N.C., 1992. Mangrove floristics and biogeography, in: Robertson, A.I., Alongi, D.M. (Eds.), Tropical Mangrove Ecosystems. American Geophysical Union, Washington D.C., pp. 63–100.

Eckert, C.G., Kalisz, S., Geber, M.A., Sargent, R., Elle, E., Cheptou, P.O., Goodwillie, C., Johnston, M.O., Kelly, J.K., Moeller, D.A., Porcher, E., Ree, R.H., Vallejo-Marín, M., Winn, A.A., 2010. Plant mating systems in a changing world. Trends Ecol. Evol. 25, 35–43. https://doi.org/10.1016/j.tree.2009.06.013

Ghazoul, J., 2005. Pollen and seed dispersal among dispersed plants. Biol. Rev. 80, 413–443. https://doi.org/10.1017/S1464793105006731

Giri, C., Ochieng, E., Tieszen, L.L., Zhu, Z., Singh, A., Loveland, T., Masek, J., Duke, N., 2011. Status and distribution of mangrove forests of the world using earth observation satellite data. Glob. Ecol. Biogeogr. 20, 154–159. https://doi.org/10.1111/j.1466-8238.2010.00584.x

Goldberg, N.A., Heine, J.N., 2017. Life on the leading edge: Phenology and demography of the red mangrove Rhizophora mangle L. at the northern limit of its expanding range. Flora 235,

76–82. https://doi.org/10.1016/j.flora.2017.09.003
González-Varo, J.P., Albaladejo, R.G., Aparicio, A., 2009. Mating patterns and spatial distribution of conspecific neighbours in the Mediterranean shrub Myrtus communis (Myrtaceae). Plant Ecol. 203, 207–215. https://doi.org/10.1007/s11258-008-9534-7
Harder, L.D., Barrett, S.C.H., 1995. Mating cost of large floral displays in hermaphrodite plants. Nature 373, 512. https://doi.org/10.1038/373512a0
Hargreaves, A.L., Eckert, C.G., 2014. Evolution of dispersal and mating systems along geographic gradients: Implications for shifting ranges. Funct. Ecol. 28, 5–21. https://doi.org/10.1111/1365-2435.12170
Hasan, S., Triest, L., Afrose, S., De Ryck, D.J.R., 2018. Migrant pool model of dispersal explains strong connectivity of Avicennia officinalis within Sundarban mangrove areas: Effect of fragmentation and replantation. Estuar. Coast. Shelf Sci. 214, 38–47. https://doi.org/10.1016/j.ecss.2018.09.007
Hermansen, T.D., Ayre, D.J., Minchinton, T.E., 2014a. Effects of stand size on pollination in temperate populations of the mangrove Avicennia marina. Plant Ecol. 215, 1153–1162. https://doi.org/10.1007/s11258-014-0374-3
Hermansen, T.D., Britton, D.R., Ayre, D.J., Minchinton, T.E., 2014b. Identifying the real pollinators? Exotic honeybees are the dominant flower visitors and only effective pollinators of Avicennia marina in Australian temperate mangroves. Estuaries and Coasts 37, 621–635. https://doi.org/10.1007/s12237-013-9711-3
Hermansen, T.D., Minchinton, T.E., Ayre, D.J., 2017. Habitat fragmentation leads to reduced pollinator visitation, fruit production and recruitment in urban mangrove forests. Oecologia 185, 221–231. https://doi.org/10.1007/s00442-017-3941-1
Hermansen, T.D., Roberts, D.G., Toben, M., Minchinton, T.E., Ayre, D.J., 2015. Small Urban Stands of the Mangrove Avicennia marina are Genetically Diverse but Experience Elevated Inbreeding. Estuaries and Coasts 38, 1898–1907. https://doi.org/10.1007/s12237-015-9955-1
Jombart, T., Ahmed, I., 2011. adegenet 1.3-1: New tools for the analysis of genome-wide SNP data. Bioinformatics 27, 3070–3071. https://doi.org/10.1093/bioinformatics/btr521
Jombart, T., Devillard, S., Balloux, F., 2010. Discriminant analysis of principal components: A new method for the analysis of genetically structured populations. BMC Genet. 11, 94. https://doi.org/10.1186/1471-2156-11-94
Kalisz, S., Vogler, D.W., Hanley, K.M., 2004. Context-dependent autonomous self-fertilization yields reproductive assurance and mixed mating. Nature 430, 884–887. https://doi.org/10.1038/nature02776
Kameyama, Y., Kudo, G., 2015. Intrinsic and extrinsic factors acting on the reproductive process in alpine-snowbed plants: Roles of phenology, biological interaction, and breeding system. Plant Species Biol. 30, 3–15. https://doi.org/10.1111/1442-1984.12070
Kangas, P.C., Lugo, A.E., 1990. The distribution of mangroves and saltmarsh in Florida. Trop. Ecol. 31, 32–39.
Kelleway, J.J., Cavanaugh, K., Rogers, K., Feller, I.C., Ens, E., Doughty, C., Saintilan, N., 2017. Review of the ecosystem service implications of mangrove encroachment into salt marshes. Glob. Chang. Biol. 23, 3967–3983. https://doi.org/10.1111/gcb.13727
Kennedy, J.P., Preziosi, R.F., Rowntree, J.K., Feller, I.C., 2020a. Is the central-marginal hypothesis a general rule? Evidence from three distributions of an expanding mangrove species, Avicennia germinans (L.) L. Mol. Ecol. https://doi.org/10.1111/mec.15365

Kennedy, J.P., Preziosi, R.F., Rowntree, J.K., Feller, I.C., 2020b. Data from: Is the central-marginal hypothesis a general rule? Evidence from three distributions of an expanding mangrove species, Avicennia germinans (L.) L. Dryad Digit. Repos. https://doi.org/10.5061/dryad.69p8cz8xh

Landry, C.L., 2013. Pollinator-mediated competition between two co-flowering Neotropical mangrove species, Avicennia germinans (Avicenniaceae) and Laguncularia racemosa (Combretaceae). Ann. Bot. 111, 207–214. https://doi.org/10.1093/aob/mcs265

Lonard, R.I., Judd, F.W., Summy, K., DeYoe, H., Stalter, R., 2017. The Biological Flora of Coastal Dunes and Wetlands: Avicennia germinans (L.) L. J. Coast. Res. 33, 191–207. https://doi.org/10.2112/07-0933.1

Maguire, T., Saenger, P., Baverstock, P., Henry, R., 2000. Microsatellite analysis of genetic structure in the mangrove species Avicennia marina (Forsk.) Vierh.(Avicenniaceae). Mol. Ecol. 9, 1853–1862. https://doi.org/10.1046/j.1365-294x.2000.01089.x

Mitchell, R.J., Karron, J.D., Holmquist, K.G., Bell, J.M., 2004. The influence of Mimulus ringens floral display size on pollinator visitation patterns. Funct. Ecol. 18, 116–124. https://doi.org/10.1111/j.1365-2435.2004.00812.x

Moeller, D.A., Briscoe Runquist, R.D., Moe, A.M., Geber, M.A., Goodwillie, C., Cheptou, P.O., Eckert, C.G., Elle, E., Johnston, M.O., Kalisz, S., Ree, R.H., Sargent, R.D., Vallejo-Marin, M., Winn, A.A., 2017. Global biogeography of mating system variation in seed plants. Ecol. Lett. 20, 375–384. https://doi.org/10.1111/ele.12738

Moeller, D.A., Geber, M.A., Eckhart, V.M., Tiffin, P., 2012. Reduced pollinator service and elevated pollen limitation at the geographic range limit of an annual plant. Ecology 93, 1036–1048. https://doi.org/10.1890/11-1462.1

Morgan, M.T., Wilson, W.G., Knight, T.M., 2005. Plant population dynamics, pollinator foraging, and the selection of self-fertilization. Am. Nat. 166, 169–183. https://doi.org/10.1086/431317

Mori, G.M., Zucchi, M.I., Sampaio, I., Souza, A.P., 2010. Microsatellites for the mangrove tree Avicennia germinans (Acanthaceae): Tools for hybridization and mating system studies. Am. J. Bot. 97, 79–81. https://doi.org/10.3732/ajb.1000219

Mori, G.M., Zucchi, M.I., Souza, A.P., 2015. Multiple-geographic-scale genetic structure of two mangrove tree species: The roles of mating system, hybridization, limited dispersal and extrinsic factors. PLoS One 10, e0118710. https://doi.org/10.1371/journal.pone.0118710

Nadia, T.D.L., De Menezes, N.L., Machado, I.C., 2013. Floral traits and reproduction of Avicennia schaueriana Moldenke (Acanthaceae): A generalist pollination system in the Lamiales. Plant Species Biol. 28, 70–80. https://doi.org/10.1111/j.1442-1984.2011.00361.x

Neal, P.R., Anderson, G.J., 2005. Are “mating systems” “breeding systems” of inconsistent and confusing terminology in plant reproductive biology? Or is it the other way around? Plant Syst. Evol. 250, 173–185. https://doi.org/10.1007/s00606-004-0229-9

Nettel-Hernanz, A., Dodd, R.S., Ochoa-Zavala, M., Tovilla-Hernández, C., Días-Gallegos, J.R., 2013. Mating System Analyses of Tropical Populations of the Black Mangrove, Avicennia germinans (L.) L. (Avicenniaceae). Bot. Sci. 91, 115–117.

Nettel, A., Dodd, R.S., Afzal-Rafii, Z., Tovilla-Hernández, C., 2008. Genetic diversity enhanced by ancient introgression and secondary contact in East Pacific black mangroves. Mol. Ecol. 17, 2680–2690. https://doi.org/10.1111/j.1365-294X.2008.03766.x

Nettel, A., Rafii, F., Dodd, R.S., 2005. Characterization of microsatellite markers for the mangrove tree Avicennia germinans L. (Avicenniaceae). Mol. Ecol. Notes 5, 103–105.

https://doi.org/10.1111/j.1471-8286.2004.00851.x
Ochoa□Zavala, M., Jaramillo□Correa, J.P., Piñero, D., Nettel□Hernanz, A., Núñez□Farfán, J., 2019. Contrasting colonization patterns of black mangrove (Avicennia germinans (L.) L.) gene pools along the Mexican coasts. J. Biogeogr. 46, 884–898. https://doi.org/10.1111/jbi.13536
Ochoa□Zavala, M., Osorio-Olvera, L., Piñero, D., Núñez-Farfán, J., 2020. Inferring potential barriers to gene flow in tropical populations of Avicennia germinans. Aquat. Bot. 161, 103170. https://doi.org/10.1016/j.aquabot.2019.103170
Osland, M.J., Feher, L.C., Griffith, K.T., Cavanaugh, K.C., Enwright, N.M., Day, R.H., Stagg, C.L., Krauss, K.W., Howard, R.J., Grace, J.B., Rogers, K., 2017. Climatic controls on the global distribution, abundance, and species richness of mangrove forests. Ecol. Monogr. 87, 341–359. https://doi.org/10.1002/ecm.1248
Osland, M.J., Feher, L.C., López-Portillo, J., Day, R.H., Suman, D.O., Guzmán Menéndez, J.M., Rivera-Monroy, V.H., 2018. Mangrove forests in a rapidly changing world: Global change impacts and conservation opportunities along the Gulf of Mexico coast. Estuar. Coast. Shelf Sci. 214, 120–140. https://doi.org/10.1016/j.ecss.2018.09.006
Pannell, J.R., 2015. Evolution of the mating system in colonizing plants. Mol. Ecol. 24, 2018–2037. https://doi.org/10.1111/mec.13087
Pecl, G.T., Araújo, M.B., Bell, J.D., Blanchard, J., Bonebrake, T.C., Chen, I.C., Clark, T.D., Colwell, R.K., Danielsen, F., Evengård, B., Falconi, L., Ferrier, S., Frusher, S., Garcia, R.A., Griffis, R.B., Hobday, A.J., Janion-Scheepers, C., Jarzyna, M.A., Jennings, S., Lenoir, J., Linnetved, H.I., Martin, V.Y., McCormack, P.C., McDonald, J., Mitchell, N.J., Mustonen, T., Pandolfi, J.M., Pettorelli, N., Popova, E., Robinson, S.A., Scheffers, B.R., Shaw, J.D., Sorte, C.J.B., Strugnell, J.M., Sunday, J.M., Tuanmu, M.N., Vergés, A., Villanueva, C., Wernberg, T., Wapstra, E., Williams, S.E., 2017. Biodiversity redistribution under climate change: Impacts on ecosystems and human well-being. Science (80-.). 355, eaai9214. https://doi.org/10.1126/science.aai9214
Peñuelas, J., Boada, M., 2003. A global change-induced biome shift in the Montseny mountains (NE Spain). Glob. Chang. Biol. 9, 131–140. https://doi.org/10.1046/j.1365-2486.2003.00566.x
Peterson, M.L., Kay, K.M., 2015. Mating system plasticity promotes persistence and adaptation of colonizing populations of hermaphroditic angiosperms. Am. Nat. 185, 28–43. https://doi.org/10.5061/dryad.n5rd6
Primack, R., Duke, N., Tomlinson, P.B., 1981. Floral morphology in relation to pollination ecology in five Queensland coastal plants. Austrobaileya 1, 346–355.
R Core Team, 2013. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Website: R-project.org.
Raju, A.J.S., Rao, P.V.S., Kumar, R., Mohan, S.R., 2012. Pollination biology of the crypto-viviparous Avicennia species (Avicenniaceae). J. Threat. Taxa 4, 3377–3389. https://doi.org/10.11609/jott.o2919.3377-89
Ritland, K., 2002. Systems Using N Independent Loci. Heredity (Edinb). 88, 221–228. https://doi.org/10.1038/sj/hdy/6800029
Rodriguez, W., Feller, I.C., Cavanaugh, K.C., 2016. Spatio-temporal changes of a mangrove–saltmarsh ecotone in the northeastern coast of Florida, USA. Glob. Ecol. Conserv. 7, 245–261. https://doi.org/10.1016/j.gecco.2016.07.005
Saintilan, N., Wilson, N.C., Rogers, K., Rajkaran, A., Krauss, K.W., 2014. Mangrove expansion

738 and salt marsh decline at mangrove poleward limits. Glob. Chang. Biol. 20, 147–157.
739 https://doi.org/10.1111/gcb.12341
740 Salas-Leiva, D.E., Mayor-Durán, V.M., Toro-Perea, N., 2009. Genetic diversity of the black
741 mangrove (Avicennia germinans L.) in Colombia. Aquat. Bot. 91, 187–193.
742 https://doi.org/10.1016/j.aquabot.2009.06.001
743 Sánchez-Núñez, D.A., Mancera-Pineda, J.E., 2012. Pollination and fruit set in the main
744 neotropical mangrove species from the Southwestern Caribbean. Aquat. Bot. 103, 60–65.
745 https://doi.org/10.1016/j.aquabot.2012.06.004
746 Sandoval-Castro, E., Dodd, R.S., Riosmena-Rodriǵuez, R., Enríquez-Paredes, L.M., Tovilla-
747 Hernández, C., López-Vivas, J.M., Aguilar-May, B., Muñiz-Salazar, R., 2014. Post-glacial
748 Expansion and Population Genetic Divergence of Mangrove Species Avicennia germinans
749 (L.) Stearn and Rhizophora mangle L. along the Mexican coast. PLoS One 9, e93358.
750 https://doi.org/10.1371/journal.pone.0093358
751 Simpson, L.T., Stein, C.M., Osborne, T.Z., Feller, I.C., 2019. Mangroves dramatically increase
752 carbon storage after 3 years of encroachment. Hydrobiologia 834, 13–26.
753 https://doi.org/10.1007/s10750-019-3905-z
754 Spalding, M., Kainuma, M., Collins, L., 2010. World atlas of mangroves. Earthscan, London,
755 UK.
756 Tomlinson, P.B., 1986. The Botany of Mangroves. Cambridge University Press, Cambridge, UK.
757 Vamosi, J.C., Knight, T.M., Steets, J.A., Mazer, S.J., Burd, M., Ashman, T.-L., 2006. Pollination
758 decays in biodiversity hotspots. Proc. Natl. Acad. Sci. 103, 956–961.
759 https://doi.org/10.1073/pnas.0507165103
760 Wickham, H., 2011. ggplot2. Wiley Interdiscip. Rev. Comput. Stat. 3, 180–185.
761 https://doi.org/10.1002/wics.147
762 Yin, G., Barrett, S.C.H., Luo, Y.B., Bai, W.N., 2016. Seasonal variation in the mating system of
763 a selfing annual with large floral displays. Ann. Bot. 117, 391–400.
764 https://doi.org/10.1093/aob/mcv186
765

Highlights

- Range expansion of foundation species may lead to shifts in their mating systems.
- Subtropical Florida *Avicennia germinans* exhibited predominant self-fertilization.
- Outcrossing did not systematically decline towards the Florida range limit.
- Outcrossing increased with conspecific cover at the Florida range margin.
- Ecological structure may impact mangrove mating systems at varying spatial scales.

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: